Journal of the American College of Cardiology
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Published by Elsevier Science Inc.

Vol. 33, No. 2, 1999
ISSN 0735-1097/99/$20.00
PII S0735-1097(98)00573-7



# Relationship Between Endothelin-1 Extraction in the Peripheral Circulation and Systemic Vascular Resistance in Patients With Severe Congestive Heart Failure

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**OBJECTIVES** This study was done to determine the spillover and extraction of endothelin-1 (ET-1) in the peripheral circulation, and to evaluate the factors that regulate local ET-1 extraction in the peripheral circulation in patients with congestive heart failure (CHF).

**BACKGROUND** The relationship between the spillover and extraction of the ET-1 in the peripheral circulation and systemic vascular resistance (SVR) has not been fully clarified.

**METHODS** We measured plasma levels of ET-1 both in femoral artery (FA) and femoral vein (FV) in 93 patients with CHF.

**RESULTS** Plasma ET-1 was significantly higher in FV than in FA in New York Heart Association (NYHA) functional class II patients, but there was no difference of ET-1 between FA and FV in functional class III patients. In patients with functional class IV, plasma ET-1 was significantly lower in FV than in FA, and SVR was significantly higher than in patients with NYHA class II or class III. Moreover, a significant positive correlation existed between plasma ET-1 extraction across the lower leg and SVR in these patients. Among the various neurohumoral factors and hemodynamics, plasma levels of ET-1, angiotensin II in the FA showed an independent and significant relationship with the plasma arteriovenous difference of ET-1 in the lower limb.

**CONCLUSIONS** Circulating ET-1 is extracted in peripheral circulation in patients with severe CHF, suggesting the possibility of upregulation of ET receptors of vascular beds in the lower limb in these patients. The peripheral extraction of ET-1 correlates with SVR in severe CHF patients and is mainly regulated by the local ET-1 and renin angiotensin systems. (J Am Coll Cardiol 1999;33:530–7) © 1999 by the American College of Cardiology

Previous studies have indicated that in many patients with chronic congestive heart failure (CHF), an increase in systemic vascular resistance (SVR) was associated with increased levels of vasoconstrictive neurohumoral factors including endothelin-1 (ET-1) (1), suggesting an important role in the pathophysiology of CHF (2–9). The ET-1 is produced and secreted by the endothelial cells of various organs and acts as both a circulating hormone and a local hormone involved in paracrine and autocrine systems. Although previous reports including our study stated that the main source of the increased ET-1 is pulmonary circulation and local ET-1 production in the lung correlates with the pulmonary vascular resistance in patients with CHF (5,6), whether ET-1 is produced or extracted from the peripheral vascular tissue and the relationship between the spillover and extraction of the ET-1 in the peripheral circulation and SVR in these patients remain unknown.

Numerous studies on the pharmacologic effects of ET-1 suggest that it plays an important pathophysiologic role in systemic and pulmonary vasoconstriction (10–14). Recent studies have shown that anti-ET-1 drugs decrease the peripheral vascular resistance and blood pressure in both normal subjects and in patients with CHF (15–17). More recently, bosentan, an endothelin-receptor antagonist, significantly lowered blood pressure in patients with hypertension (18), suggesting an important role for the local ET-1 system as an autocrine and paracrine factor in the regulation of blood pressure and peripheral vascular resistance in humans. Angiotensin II, norepinephrine, and ET-1 can directly or indirectly increase ET-1 production from the

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Manuscript received June 24, 1998; revised manuscript received August 31, 1998, accepted October 22, 1998.

**Abbreviations and Acronyms**

ACE = angiotensin-converting enzyme
ANP = atrial natriuretic peptide
BNP = brain natriuretic peptide
CHF = congestive heart failure
ET-1 = endothelin-1
FA = femoral artery
FV = femoral vein
NYHA = New York Heart Association
SVR = systemic vascular resistance

vascular tissue (19–21), and these vasoconstrictors are increased in the plasma in CHF, but the effects of these endogenous vasoconstrictors on the local ET-1 spillover or extraction remain unknown in CHF patients before and after the treatments with angiotensin-converting enzyme (ACE) inhibitors.

Although anti-ET-1 drugs decrease systemic vascular resistance in patients with CHF (15,19), whether ET-1 is produced or extracted in the peripheral vascular tissue in humans remains unknown. Therefore, we examined the spillover and extraction of the ET-1 in the lower limb circulation and the relationship between the spillover and extraction of the plasma ET-1 in the peripheral circulation and SVR in patients with CHF, and we evaluated the factors that regulate local ET-1 clearance in the peripheral circulation.

## METHODS

**Patients.** The subjects were 93 patients with symptomatic left ventricular dysfunction without renal failure who underwent cardiac catheterization for clinical indications. We also selected 15 age-matched normal subjects who, admitted complaining of chest pain, proved to be normal by coronary angiography. Informed consent was obtained from all patients before participation in the study, and the protocol was approved by the Human Investigations Committee of our institution. The subjects were 69 men and 24 women ranging in age from 17 to 79 years (mean: 59 years); 55 patients had suffered a myocardial infarction more than 3 months before the study, 25 had dilated cardiomyopathy, and 10 had hypertensive heart disease. Forty-six patients were classified according to the standards of the New York Heart Association (NYHA) as functional class II, 23 patients as class III, and 24 patients as class IV. At the entry of the study, 68 patients were treated with diuretics, 48 with ACE inhibitors, 48 with digitalis, 65 with vasodilators, and 12 with beta-blockers. All drug treatments were discontinued at least 12 h before start of the study. To evaluate the effects of ACE inhibitors on the plasma levels of ET-1, 15 functional class IV patients underwent repeated right cardiac catheterization more than 4 weeks after the study with symptoms improved.

**Study protocol.** All patients were premedicated with an oral dose of diazepam (5 mg) and rested in bed in the supine position for at least 20 min. Right-sided cardiac catheterization was performed using a 7F Swan-Ganz catheter. The heart rate was monitored by electrocardiography. Blood samples for measuring plasma ET-1 were collected simultaneously from the right femoral artery (FA) and femoral vein (FV). Blood samples for measuring the plasma levels of norepinephrine, angiotensin II, atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP) were also drawn from the right FA. A Swan-Ganz catheter was inserted through the right FV into the main pulmonary artery, where the pressure was measured. The catheter was then advanced into the pulmonary artery, and the pulmonary capillary wedge pressure was measured by inflating the balloon. Cardiac output was determined by the thermodilution method immediately after blood collection. Left ventriculography was performed using contrast medium or a radioisotope before or 1 week after the hemodynamic measurements and blood sampling. To evaluate the effects of ACE inhibitors on the plasma levels of ET-1, 15 functional class IV patients underwent repeated right cardiac catheterization and blood samplings more than 4 weeks after the study with symptoms improved.

**Measurement of immunoreactive ET-1 and other vasoconstrictor factors.** Blood for the measurement of plasma ET-1 levels was transferred to a chilled tube containing EDTA (1 mg/ml) and aprotinin (500 kallikrein inactivator units/ml), and then centrifuged at 3,000 rpm for 15 min at 4°C. The plasma thus obtained was stored at −30°C until assayed. The ET-1 was extracted by mixing 2 ml of plasma with 0.4 ml of 60% methanol containing 0.25 mg/ml Preparative C18 (Waters Chromatography Division, Mitford, Massachusetts). The recovery rate was calculated to be 72 ± 3.9% (n = 5) by $^{125}$I ET-1 with this method. The plasma ET-1 level was determined using an antibody directed against synthetic ET-1 (Peninsula Laboratories, Belmont, California) and $^{125}$I ET-1 (Amersham Japan, Tokyo) as previously reported (5). This antibody showed 100% cross-reactivity with ET-1, 7% with endothelin-2, 7% with endothelin-3, and 17% with human big ET-1. However, it did not cross-react with angiotensin I or angiotensin II, vasopressin, or human cardiac natriuretic peptides. The minimum detectable level of ET-1 was 0.5 pg/tube, the interassay coefficient of variation was 13% (n = 10), and the intraassay coefficient of variation was 11% (n = 9).

Blood for measurement of the plasma levels of norepinephrine and angiotensin II was transferred to a chilled tube containing EDTA (1 mg/ml), centrifuged at 3,000 rpm for 15 min at 4°C, and the plasma thus obtained was stored at −30°C until assayed. Plasma norepinephrine concentration was measured by high-performance liquid chromatography. Plasma angiotensin II levels were measured by a radioimmunoassay using a specific antibody directed against synthetic angiotensin II (Special Research Laboratory, Tokyo,

**Table 1.** Hemodynamic Data

| | HR (beats/min) | LVEF (%) | MBP (mm Hg) | MPA (mm Hg) | PCWP (mm Hg) | CI (l/min/m$^2$) | SVR (dyne·s·cm$^{-5}$) |
|---|---|---|---|---|---|---|---|
| NYHA II (n = 46) | 72 ± 1.8 | 36 ± 1.1 | 87 ± 2.2 | 13.7 ± 0.4 | 7.2 ± 0.5 | 2.8 ± 0.1 | 1603 ± 74 |
| NYHA III (n = 23) | 84 ± 4.5‖ | 32 ± 2.4 | 89 ± 3.9 | 20.3 ± 2.1 | 12 ± 1.2§ | 2.6 ± 0.1 | 1740 ± 145 |
| NYHA IV (n = 24) | 92 ± 2.8‡ | 30 ± 2.2* | 85 ± 4.0 | 29.2 ± 2.4‡** | 20.9 ± 1.5‡** | 2.3 ± 0.2* | 2101 ± 151†¶ |

*$p < 0.05$ vs. NYHA II. †$p < 0.01$ vs. NYHA II. ‡$p < 0.001$ vs. NYHA II. §$p < 0.05$ vs. NYHA II. ‖$p < 0.01$ vs. NYHA II. ¶$p < 0.05$ vs. NYHA III. **$p < 0.001$ vs. NYHA III with ANOVA by the Scheffé F test.

CI = cardiac index; HR = heart rate; LVEF = left ventricular ejection fraction; MPA = mean pulmonary arterial pressure; PCWP = pulmonary capillary wedge pressure; MBP = mean arterial blood pressure; SVR = systemic vascular resistance; NYHA = New York Heart Association.

Japan) as previously reported (5). This antibody had 100% cross-reactivity with angiotensin II, 0.3% cross-reactivity with angiotensin I, and no cross-reactivity with ET-1, vasopressin, and cardiac natriuretic peptides.

Samples for the assay of the plasma ANP and BNP concentrations were transferred to chilled disposable tubes containing aprotinin (500 kallikrein inactivator units/ml). The blood samples were immediately placed on ice and centrifuged at 4°C. Plasma ANP concentrations were measured with a specific immunoradiometric assay for α-human ANP using a commercial kit (Shionoria, Japan) as previously reported (22).

Briefly, this assay uses two monoclonal antibodies against α-human ANP, one recognizing a carboxyterminal sequence and the other the ring structure of ANP, and measures α-human ANP by sandwiching it between the two antibodies without plasma extraction. The minimal detectable quantity of α-human ANP is 5 pg/ml. The intraassay and interassay coefficients of variation were 5.1% and 5.8%, respectively. This assay system did not cross-react with angiotensin I or angiotensin II, vasopressin, or human BNP. The cross-reactivity with human BNP was <0.001% on a molar basis.

Plasma BNP concentrations were measured with a specific immunoradiometric assay for human BNP using a commercial kit (Shionoria, Japan) as previously reported (22). Briefly, this assay uses two monoclonal antibodies against human BNP, one recognizing a carboxyterminal sequence and the other the ring structure of BNP, respectively, and measures BNP by sandwiching it between the two antibodies without plasma extraction. The minimal detectable quantity of human BNP is 2 pg/ml. The intraassay and interassay coefficients of variation were 5.2% and 6.1%, respectively. This assay system did not cross-react with angiotensin I or angiotensin II, vasopressin, or human ANP. The cross-reactivity with human ANP was <0.001% on a molar basis.

**Calculations.** The mean arterial blood pressure and SVR were calculated from standard formulas.

**Statistical analysis.** All results were expressed as the mean ± SEM. Comparisons between multiple groups were determined by one-way analysis of variance (ANOVA) with the Scheffé F test. Univariate and stepwise multivariate linear regression analyses were used to detect independent predictors of plasma ET-1 extraction in peripheral circulation among the 18 variables (age, NYHA functional class, heart rate, mean arterial blood pressure, cardiac index, pulmonary capillary wedge pressure, left ventricular ejection fraction, SVR, ANP, BNP, norepinephrine, ET-1, angiotensin II, and treatments such as diuretics, digitalis, angiotensin converting enzyme inhibitors, vasodilators, and beta-blockers). Linear regression analysis was used to determine the relationship between continuous variables. Statistical analyses were done by use of a commercial computer software package (StatView-J4.02: Abacus Concepts). A p value <0.05 was regarded as significant.

## RESULTS

**Hemodynamic data.** Hemodynamic data are shown in Table 1. The cardiac index and the left ventricular ejection fraction were significantly lower in functional class IV patients than in functional class II patients. In contrast, pulmonary capillary wedge pressure increased with the severity of functional class. The SVR was significantly higher in functional class IV than in class II or class III patients.

**Neurohumoral data.** Vasodilative cardiac natriuretic peptides such as ANP and BNP increased with the severity of functional class. Plasma norepinephrine was significantly increased and angiotensin II was slightly increased in functional class IV patients (see Table 2).

**Plasma concentrations of ET-1 in the lower limb circulation: Comparison with the severity of heart failure.** In normal subjects, plasma ET-1 significantly increased from FA to FV (1.67 ± 0.15 vs. 2.05 ± 0.13, p = 0.003). Figure 1 shows the plasma ET-1 levels in FA and FV in patients with CHF. Plasma ET-1 levels significantly increased with the severity of CHF. Plasma ET-1 significantly increased from FA to FV (2.06 ± 0.1 vs. 2.3 ± 0.1 pg/ml, p = 0.009) in NYHA class II patients. There was no difference of ET-1 between FA and FV in functional class III patients. In NYHA functional class IV patients, plasma ET-1 decreased significantly from FA to FV (6.3 ± 0.5 vs. 4.9 ± 0.5 pg/ml, p = 0.0001).

Figure 2 shows the plasma ET-1 spillover in the lower limb (ET-1 in FV −ET-1 in FA) in normal subjects and in patients with CHF. Plasma ET-1 spillover across the lower

**Table 2.** Neurohumoral Data

| | ANP (pg/ml) | BNP (pg/ml) | Norepinephrine (pg/ml) | Ang II (pg/ml) | Plasma ET-1 Spillover in Leg (pg/ml) |
|---|---|---|---|---|---|
| NYHA II (n = 46) | 81 ± 9.4 | 134 ± 20 | 284 ± 35 | 25 ± 5.4 | 0.24 ± 0.08 |
| NYHA III (n = 23) | 140 ± 22 | 291 ± 50 | 418 ± 53 | 27 ± 5.7 | −0.013 ± 0.17 |
| NYHA IV (n = 24) | 292 ± 47*† | 954 ± 125*‡ | 1192 ± 240*† | 53 ± 18 | −1.34 ± 0.3*‡ |

*$p < 0.0001$ vs. NYHA II. †$p < 0.001$ vs. NYHA III. ‡$p < 0.0001$ vs. NYHA III, with ANOVA by Scheffé F test.
ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; Ang II = angiotensin II; ET-1 = endothelin-1; plasma ET-1 spillover in leg = ET-1 in femoral vein − ET-1 in femoral artery; NYHA = New York Heart Association.

limb significantly decreased with the severity of CHF (NYHA functional class II: 0.24 ± 0.08; class III: −0.013 ± 0.17; class IV: −1.4 ± 0.3 pg/ml). There was a significant negative correlation between the functional class and the plasma ET-1 spillover in the lower limb ($r = -0.55$, $p < 0.0001$).

**Relationship between ET-1 extraction in the lower limb and SVR in patients with CHF.** No significant correlation existed between plasma ET-1 or plasma ET-1 extraction in the lower limb (ET-1 in FA − ET-1 in FV) and SVR in patients with functional class II or class III. Although there was no significant correlation between the plasma ET-1 in FA and SVR, plasma ET-1 extraction in the lower limb significantly correlated with SVR in functional class IV patients (Fig. 3).

**Relationship between plasma ET-1 extraction in the lower limb circulation and clinical characteristics, treatments, hemodynamics and neurohumoral factors.** Table 3 shows the results of univariate and multivariate analyses to assess the factors regulating the ET-1 extraction in lower limb circulation in 93 patients with CHF. By univariate analyses, 10 variables such as functional class, and neurohumoral and hemodynamic variables, were significant predictors of the plasma ET-1 extraction in the lower limb circulation. According to stepwise multivariate analyses, only high levels of the plasma arterial ET-1 ($p < 0.0001$), angiotensin II ($p = 0.025$) and SVR ($p = 0.0079$) were significant independent predictors of the plasma ET-1 extraction in the lower limb. Figure 4 shows the correlation between the plasma levels of arterial ET-1 and the plasma ET-1 extraction in the lower limb circulation by linear regression analysis ($r = 0.705$, $p < 0.0001$).

**Plasma concentrations and extraction of ET-1 in the peripheral circulation: Effects of ACE inhibitors.** Figure 5 shows the plasma ET-1 levels in FA and FV before and after administration of ACE inhibitors. Plasma ET-1 levels decreased significantly from FA to FV in NYHA class IV patients. After the treatment with ACE inhibitors, plasma levels of ET-1 in both the FA and the FV significantly decreased concomitant with the significant improvement of symptoms. The plasma ET-1 extraction across the lower limb significantly decreased (normalized) after treatment with ACE inhibitors.

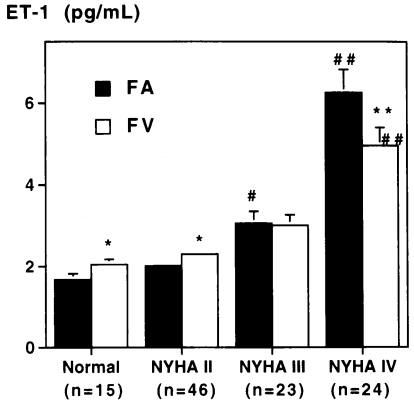


**Figure 1.** Plasma levels of endothelin-1 (ET-1) in age-matched normal subjects and in patients with congestive heart failure. FA = femoral artery; FV = femoral vein. **Closed columns** represent the ET-1 level in FA and **open columns** represent the ET-1 level in FV. *$p < 0.01$, **$p < 0.001$ vs. the value of the ET-1 level in FA. #$p < 0.05$ vs. the value of the ET-1 level of NYHA functional class II patients. ##$p < 0.0001$ vs. the value of the ET-1 level of NYHA functional class II or class III patients with ANOVA by the Scheffé F test.

## DISCUSSION

We demonstrated that local ET-1 spillover in the lower limb occurred in normal subjects and in mild CHF patients (NYHA functional class II) and ET-1 extraction was predominant in patients with severe CHF (NYHA functional class IV) concomitant with the significant increase of SVR. Although plasma ET-1 levels increased in relation to the severity of CHF as previously reported (2–5), the plasma ET-1 spillover across the lower limb significantly decreased with the severity of CHF in contrast to the significant increase of the difference across the lung in the pulmonary circulation as previously reported (data not shown) (5,6). These findings indicate differential regulation of ET-1 spillover or extraction in the lung and the peripheral circulation (such as the lower limb) in patients with CHF. To our knowledge, the only report about arteriovenous ET-1 extraction in patients with CHF was of renal extraction detected for the difference of plasma ET-1 between the aorta and renal vein (23); however, the observation of those investigators was partly due to the glomerular filtration of ET-1. Therefore, we showed, for the first time, plasma

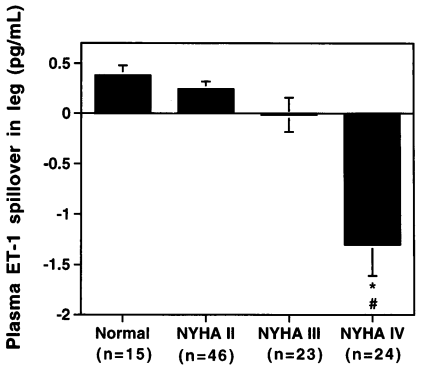


**Figure 2.** Plasma endothelin-1 (ET-1) spillover in the leg in age-matched normal subjects and in patients with congestive heart failure. Plasma ET-1 spillover in the leg = ET-1 in femoral vein − ET-1 in femoral artery. *$p < 0.0001$ vs. the value of NYHA class II. #$p < 0.0001$ vs. the value of NYHA class III with ANOVA by the Scheffé F test.

ET-1 was extracted in vascular beds in NYHA functional class IV patients. Moreover, whether ET receptors are upregulated or downregulated in CHF remains controversial; our present study supports upregulation of ET receptors in the vascular tissue of lower limb in patients with severe CHF.

Previous studies, including one by Dupis et al. (24), indicated that various organs rich in endothelial cells including peripheral vascular tissue are also important sites for clearance and production of ET-1 in patients with CHF, in which ET-1 production is generally activated. The present study suggests the balance of production and clearance in the peripheral circulation differs with the severity of CHF. Moreover, the ET-1 extraction in functional class IV patients was normalized after the improvement of symptoms by ACE inhibitors. Indeed, Clavell et al. (25) recently reported that the circulating and local ET-1 levels decreased after treatment with ACE inhibitors in association with a decrease in the systemic vascular resistance in a model of low cardiac output CHF in dogs, suggesting the contribution of the renin angiotensin system to the activation of the local ET-1 production.

According to stepwise multivariate analyses, only high levels of plasma arterial ET-1, angiotensin II and SVR were significant independent predictors of the plasma ET-1 extraction in the lower limb circulation in the patients with CHF, suggesting the local ET-1 extraction in peripheral vascular tissue was mainly regulated by the local ET-1 and renin angiotensin systems. These findings are consistent with the role of local endogenous ET-1 in the peripheral circulation in the pathophysiology of CHF. Because angiotensin II and ET-1 stimulate the ET-1 production in vitro (19,20) and in vivo (21), the total amount of ET-1 production in the peripheral vascular tissue may increase with the severity of CHF.

Moreover, angiotensin II, which is increased in plasma of CHF patients, induces upregulation of ET type A receptor as well as its mRNA in human vascular smooth muscle cell in vitro (26), and the findings of these investigators support our data that plasma angiotensin II in FA independently correlated with plasma arteriovenous ET-1 extraction in the lower circulation, which may indicate upregulation of ET receptors. Indeed, Schiffrin et al. (27) recently reported that the ET-1 mRNA was abundant in the small arteries of subcutaneous gluteal fat in moderate to severe hypertensive patients, suggesting that the amount of total ET-1 production in the lower limb vascular tissue increases with the severity of CHF.

In the present study, the plasma venous ET-1 level decreased relative to the arterial ET-1 level in the lower limb in patients with severe CHF (NYHA class IV), in which ET-1 production is generally activated at the tissue level. Recently, Sakai et al. (28) reported that ET-1 receptor antagonist improved long-term survival in rats with heart failure in which the ET-A receptor was upregulated. These findings suggest the local activity of ET-1 in the lower limb vascular tissue is enhanced in patients with severe CHF. This is attributed not only to the increase in the production of ET-1 but also to the upregulation of ET-receptors.

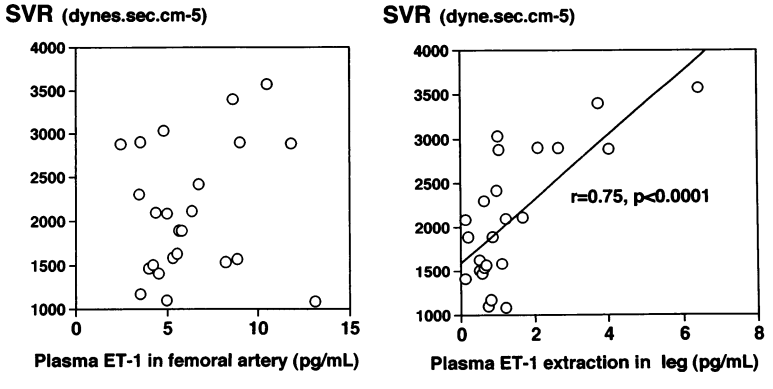


**Figure 3.** Relationship between the plasma arterial endothelin-1 (ET-1) level and plasma ET-1 extraction in the lower limb and systemic vascular resistance (SVR) in NYHA functional class IV patients. Plasma ET-1 extraction in the leg = ET-1 in femoral artery −ET-1 in femoral vein.

**Table 3.** Univariate and Multivariate Linear Model of Plasma Endothelin-1 Extraction in the Lower Limb in Patients With CHF

| Variable | Univariate Corrrelation Coefficient | p-Value | Multivariate Beta Coefficient | p-Value |
|---|---|---|---|---|
| Age (year) | 0.073 | NS | | |
| NYHA (functional class) | 0.507 | < 0.0001 | | |
| Diuretics (treatment = 1; no treatment = 0) | 0.158 | NS | | |
| Digitalis (treatment = 1; no treatment = 0) | 0.195 | NS | | |
| ACEI (treatment = 1; no treatment = 0) | 0.058 | NS | | |
| Vasodilators (treatment = 1; no treatment = 0) | −0.085 | NS | | |
| $\beta$-blockers (treatment = 1; no treatment = 0) | −0.05 | NS | | |
| Heart rate (beats/min) | 0.347 | 0.0006 | | |
| Mean arterial blood pressure (mm Hg) | 0.024 | NS | | |
| Cardiac index (l/min/m$^2$) | −0.203 | NS | | |
| Pulmonary capillary wedge pressure (mm Hg) | 0.467 | < 0.0001 | | |
| Left ventricular ejection fraction (%) | −0.219 | 0.0173 | | |
| Systemic vascular resistance (dyne·s·cm$^{-5}$) | 0.382 | < 0.0001 | 0.00034 | 0.0079 |
| Atrial natriuretic peptide (pg/ml) | 0.529 | < 0.0001 | | |
| Brain natriuretic peptide (pg/ml) | 0.459 | < 0.0001 | | |
| Norepinephrine (pg/ml) | 0.508 | < 0.0001 | | |
| Endothelin-1 (pg/ml) | 0.705 | < 0.0001 | 0.302 | < 0.0001 |
| Angiotensin II (pg/ml) | 0.382 | 0.0004 | 0.0036 | 0.025 |

After a stepwise multivariate linear regression analysis by the procedure of forward selection of variables (the F value for entering a variable into the model is 4.00), only three variables as listed were selected and the other 15 variables were not selected.

These factors may contribute to the significant increase of systemic vascular resistance in patients with NYHA class IV owing to a significant positive correlation between plasma ET-1 extraction in the peripheral circulation and SVR in these patients (Fig. 3).

Because cardiac natriuretic peptides, which are increased in CHF, have been reported to decrease the angiotensin II-stimulated ET-1 production by endothelial cells by increasing the level of second messenger, cyclic guanosine monophosphate (20), we hypothesized that the arterial levels of ANP and BNP modulate the ET-1 spillover in patients with CHF. However, the cardiac natriuretic peptide level did not independently influence the plasma ET-1 extraction in the present study, suggesting that the endogenous cardiac natriuretic peptides are not the main factor regulating ET-1 secretion in the lower limb circulation and the cardiac natriuretic peptide receptor coupled to guanylate cyclase may be downregulated in the peripheral circulation in patients with severe CHF as previously reported (22,29).

**Clinical implications.** Our findings indicate that the plasma venous ET-1 level is underestimated compared to the plasma arterial ET-1 level probably because of its increased extraction in severe CHF. Moreover, the significant extraction of ET-1 in the lower limb suggests the possibility of upregulation of ET receptors in patients with severe CHF (28), indicating the usefulness of ET receptor antagonists in these patients.

Our present finding that the changes in the plasma levels of ET-1 concomitant with the normalization of local ET-1 extraction before and after the treatments of ACE inhibitors suggests an important local interaction between the renin angiotensin system and endothelin system in patients with CHF.

Patients with the highest plasma ET-1 levels during maximal exercise have been found to have the greatest reductions in exercise capacity, perhaps by limiting exercise-induced vasodilation (30,31); interestingly, the changes in plasma ET-1 levels have been found to be a sensitive predictor of functional parameters, such as NYHA functional class and 6-min walk distance, after treatment with carvedilol, by which the mortality of patients with CHF was improved (32). Because the ability of peripheral blood vessels to dilate in response to many physiologic and pharmacologic vasodilator stimuli is markedly attenuated in patients with CHF, a significant correlation existed between the plasma ET-1 extraction in the lower limb and NYHA functional class, suggesting the plasma ET-1 extraction contributes to exercise intolerance by limiting the ability of the peripheral vasculature to dilate during exercise (30,31). Further studies are needed to evaluate the relationship between the plasma arteriovenous difference of ET-1 in the lower limbs and the exercise tolerance in patients with CHF.

**Study limitations.** Blood samples were also collected from the FA and FV to confirm the spillover or extraction of ET-1 in the lower limb circulation. We measured the plasma arteriovenous ET-1 difference, but this was not the actual ET-1 production or clearance as it is influenced by the balance of local production and clearance of ET-1 in the lower limb. In addition, we measured the cardiac output, but did not evaluate the blood flow in the lower limb. However, a significant decrease of plasma ET-1 from FA to FV in functional class IV patients is independent of the

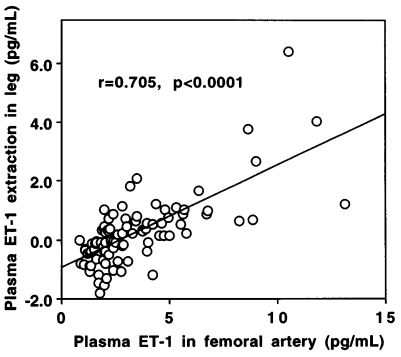


**Figure 4.** Correlation between the plasma arterial endothelin-1 (ET-1) level and plasma ET-1 extraction in the peripheral circulation in patients with congestive heart failure. Plasma ET-1 extraction in the peripheral circulation = ET-1 in femoral artery − ET-1 in femoral vein.

blood flow in the leg. It is difficult to evaluate the total amount of production and clearance of ET-1 in the peripheral circulation of CHF patients, and further studies are needed to clarify our hypothesis.

**Conclusions.** Local ET-1 spillover in the lower limb was observed in normal subjects and in mild CHF patients. However, circulating ET-1 is extracted in peripheral circulation in patients with severe CHF, suggesting the possibility of upregulation of ET receptors of vascular beds in the lower limb in patients with severe CHF. The peripheral extraction of ET-1 correlates with SVR in severe CHF patients and is mainly regulated by the local ET-1 and renin angiotensin systems in patients with CHF. Although the physiologic and pathophysiologic role of the ET-1 extraction in the lower limb remains unclear, the significant extraction of ET-1 in the lower limb suggests the possibility of upregulation of ET receptors in patients with severe CHF, indicating the usefulness of ET receptor antagonists for vasodilation of the lower limb in patients with CHF. Additional studies are needed to evaluate the relationship between the improvement of blood flow in the lower limb and the increase of exercise capacity in patients with CHF after treatment with ET receptor antagonists.

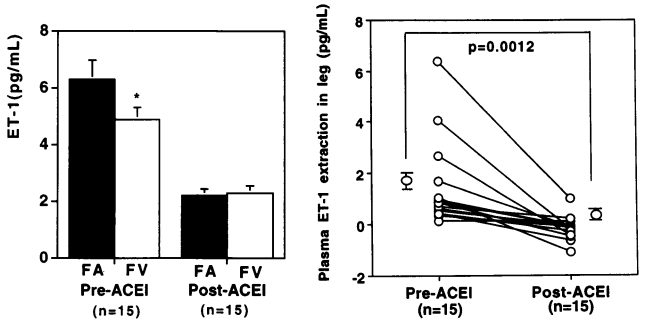


**Figure 5.** Plasma levels of endothelin-1 (ET-1) of femoral artery (FA) and femoral vein (FV) and plasma ET-1 extraction in leg before and after the treatments with angiotensin-converting enzyme inhibitors (ACEI). *$p < 0.01$ vs. the value of ET-1 in FA. Plasma ET-1 extraction in the leg = ET-1 in femoral artery − ET-1 in femoral vein.

## Acknowledgments

We wish to thank Ms. Ikuko Sakaguchi for excellent technical assistance. We also express thanks to Mr. Daniel Mrozek for assistance in preparing the manuscript. This study was supported by a Grant-in-Aid for Scientific Research in Japan.

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## REFERENCES

1. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–5.
2. Stewart DJ, Cernacek P, Costello KB, Rouleau JL. Elevated ET-1 in heart failure and loss of normal response to postural change. Circulation 1992;85:510–7.
3. Cody RJ, Haas GJ, Binkley PF, Capers Q, Kelley R. Plasma endothelin correlates with extent of pulmonary hypertension in patients with chronic congestive heart failure. Circulation 1992;85:504–9.
4. Tsutamoto T, Wada A, Maeda Y, Adachi T, Kinoshita M. Relation between endothelin-1 spillover in the lungs and pulmonary vascular resistance in patients with chronic heart failure. J Am Coll Cardiol 1994;23:1427–33.
5. Yoshibayashi M, Hirata Y, Nakao K, et al. Plasma endothelin concentrations in patients with pulmonary hypertension associated with congestive heart defects: evidence of increased production of endothelin in pulmonary hypertension. Circulation 1991;84:2280–5.
6. Packer M. Neurohormonal interactions and adaptations in congestive heart failure. Circulation 1988;77:721–9.
7. Francis GS, Goldsmith SR, Levine TB, Olivary MT, Cohn JN. The neurohormonal axis in congestive heart failure. Ann Intern Med 1984;101:370–7.
8. Cohn JN, Levine TB, Olivari MT, et al. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 1984;311:819–23.
9. Tsutamoto T, Hisanaga T, Fukai D, et al. Prognostic value of plasma soluble intercellular adhesion molecule-1 and endothelin-1 concentration in patients with chronic heart failure. Am J Cardiol 1995;76:803–8.
10. Yanagisawa M, Inoue A, Ishikawa T, et al. Primary structure, synthesis, and biological activity of rat endothelin, and endothelium-derived vasoconstrictor peptide. Proc Natl Acad Sci USA 1988;85:6964–7.
11. Miller WL, Redfield MM, Burnett JC Jr. Integrated cardiac, renal, and endocrine actions of endothelin. J Clin Invest 1989;83:317–20.
12. Vierhapper H, Wagner O, Nowotny P, Waldhausl W. Effects of ET-1 in man. Circulation 1990;81:1415–8.
13. Lerman A, Hildebrand FL, Aarhus LL, Burnett JC. Endothelin has biological actions at pathophysiological concentrations. Circulation 1991;83:1808–14.
14. Kiowski W, Luscher TF, Linder L, Buhler FR. Endothelin-1-induced vasoconstriction in humans: reversal by calcium

channel blockade but not by nitrovasodilators or endothelium-derived relaxing factor. Circulation 1991;83:469–75.
15. Kiowski W, Sutsch G, Hunziker P, et al. Evidence for endothelin-1 mediated vasoconstriction in severe chronic heart failure. Lancet 1995;346:732–6.
16. Haynes W, Ferro CJ, O'Kane KPJ, Somerville D, Lomax CC, Webb DJ. Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in humans. Circulation 1996;93:1860–70.
17. Love MP, Haynes WG, Gray GA, Webb DJ, McMurray JJV. Vasodilator effects of endothelin-converting enzyme inhibition and endothelin $ET_A$ receptor blockade in chronic heart failure patients with ACE inhibitors. Circulation 1996;94: 2131–7.
18. Krum H, Viskoper RJ, Lacourciere Y, Budde M, Charlon V, for the Bosentan Hypertension Investigators. The effect of an endothelin-receptor antagonists, bosentan, on blood pressure in patients with essential hypertension. N Engl J Med 1998; 338:784–90.
19. Imai T, Hirata Y, Emori T, Yanagisawa M, Masaki T, Marumo F. Induction of endothelin-1 gene by angiotensin and vasopressin in endothelial cells. Hypertension 1992;19: 753–7.
20. Kohno M, Yokokawa K, Horio T, Yasunari K, Murakawa K, Takeda T. Atrial and brain natriuretic peptides inhibit the endothelin-1 secretory response to angiotensin II in porcine aorta. Circ Res 1992;70:241–7.
21. Jilma B, Krejcy K, Dirnberger E, et al. Effects of angiotensin-II infusion at pressor and subpressor doses on endothelin-1 plasma levels in healthy men. Life Sci 1997;60: 1859–66.
22. Tsutamoto T, Wada A, Maeda K, et al. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: prognostic role of plasma brain natriuretic peptide in patients with chronic symptomatic left ventricular dysfunction. Circulation 1997;96:509–16.
23. McMurray JJ, Ray SG, Abdullah I, Dargie HJ, Morton J. Plasma endothelin in chronic heart failure. Circulation 1992; 85:1374–9.
24. Dupis J, Stewart DJ, Cernacek P, Gosselin G. Human pulmonary circulation is an important site for both clearance and production of endothelin-1. Circulation 1996;94:1578–84.
25. Clavell AL, Mattingly MT, Stevens TL, et al. Angiotensin-converting enzyme inhibition modulates endogenous endothelin in chronic canine thoracic inferior vena caval constriction. J Clin Invest 1996;97:1286–92.
26. Hatakeyama H, Miyamori I, Yamagishi S, Takeda Y, Takeda R, Yamamoto H. Angiotensin II up-regulates the expression of type A endothelin receptor in human vascular smooth muscle cells. Biochem Mol Biol Int 1994;34:127–34.
27. Schiffrin EL, Deng LY, Sventek P, Day R. Enhanced expression of endothelin-1 gene in resistance arteries in severe human essential hypertension. J Hypertens 1997;15: 57–63.
28. Sakai S, Miyauchi T, Kobayashi M, Yamaguchi I, Goto K, Sugishita Y. Inhibition of myocardial endothelin pathway improves long-term survival in heart failure. Nature 1996;384: 353–5.
29. Tsutamoto T, Kanamori T, Morigami N, Sugimoto Y, Yamaoka O, Kinoshita M. Possibility of downregulation of atrial natriuretic peptide receptor coupled to guanylate cyclase in peripheral vascular beds of patients with chronic severe heart failure. Circulation 1993;87:70–5.
30. Krum H, Goldsmith R, Wilshire-Clement M, Miller M, Packer M. Importance of endothelin in exercise intolerance of heart failure. Am J Cardiol 1995;75:1282–3.
31. Krum H, Gu A, Wilshire-Clement M, et al. Changes in plasma endothelin-1 levels reflect clinical response to $\beta$-blocker in chronic heart failure. Am Heart J 1996;131:337–41.
32. Packer M, Bristow MR, Cohn JN, et al. for the U.S. Carvedilol Heart Failure Study Group. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. N Engl J Med 1996;334:1349–55.